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PLANT TOLERANCE TO NICKEL TOXICITY: I. INFLUX, TRANSPORT, AND ACCUMULATION OF NICKEL IN FOUR SPECIES

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ABSTRACT: Plant tolerance to nickel (Ni) toxicity depends on plant differences for uptake and distribution within tissues. Differences among and within species for Ni tolerance/accumulation might be used to identify or develop plants for remediation of high Ni soil conditions. Solution culture experiments were conducted under controlled conditions to determine influx (IN) into roots, transport (TR) from roots to shoots, and accumulation of Ni in four plant species grown at different Ni levels. White clover (*Trifolium repens* L.) had high dry matter (DM) at high Ni levels because of its low IN and TR of Ni. At Ni levels <60 μM, maize (*Zea mays* L.) had high DM probably because of its low TR even though it had high IN of Ni. Ryegrass (*Lolium perenne* L.) was sensitive to Ni toxicity because of its high IN and TR of Ni. The sensitivity of cabbage (*Brassica oleracea* var. *capitata* L.) to Ni toxicity was correlated with high TR even though it had low IN of Ni. Nickel accumulation in shoots was relatively high for cabbage and low for maize. Maize averaged ~60-fold less Ni in shoots than cabbage and ~10-fold less than ryegrass when plants were grown with <120 μM Ni. Plant tolerance to Ni toxicity was related to low IN of Ni, and especially to TR of Ni. Selecting or developing genotypes with low TR of Ni might improve plant tolerance to moderate Ni toxicity and reduce the flow of Ni from contaminated soils to shoot organs.

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INTRODUCTION

The biological importance and essentiality of Ni to plants, animals, bacteria, and humans have been investigated and reviewed (Brown et al., 1987; Eskew et al., 1983, 1984; Klucas et al., 1983; Misha and Kar, 1974; Solomons, 1984; Welch, 1981). Nickel is an essential micronutrient for some higher plants (Brown et al., 1987; Eskew et al., 1984), animals (Solomons, 1984), and humans (Solomons, 1984) at low levels, but can be toxic to plants (Bingham et al., 1986; Farago and Cole, 1986; Foy et al., 1978), animals, and humans (Hammond and Foulkes, 1986; Nieboer and Nriagu, 1992) at high levels. The main sources of body-Ni in humans and animals are provided by food and water (Hammond and Foulkes, 1986), and Ni contamination resulting from mining, smelting operations, and disposal/recycling of municipal sewage sludge/wastes could threaten animal/human health. However, plant species and cultivars differ greatly in their response to Ni toxicity (Bingham et al., 1986; Piccini and Malavolta, 1992; Symeonidis et al., 1985), and some herbarium plants can accumulate Ni in shoots to above 1-2% of the dry weight (Baker et al., 1985; Brooks et al., 1981; Reeves, 1988). Orchardgrass (*Dactylis glomerata* L.) contained 15 times more Ni than maize when grown on the same soil (Chaney, 1983). Therefore, selecting and/or developing either Ni-accumulating plants to remediate Ni-contaminated soils or Ni-excluding plants to reduce Ni flow from soils into the food chain should be possible.

The objectives of our study were to determine the IN into roots, TR from roots to shoots, and accumulation of Ni relative to Ni tolerance in four plant species.

MATERIALS AND METHODS

Plant Culture

Four plants species [white clover (*Trifolium repens* L. cv. 'Ladino F-8339 G'), ryegrass (*Lolium perenne* L. cv 'Linn'), cabbage (*Brassica oleracea* var. capitata L. cv 'Early Jersey Wakefield'), and maize (*Zea mays* L. hybrid 'Early Sunglow')] were grown in nutrient solutions in a growth chamber. Chamber conditions were 25/20°C, 14/10 h, 60/70% relative humidity (light/dark) with a photon flux density of ~400 μE/S/m^2 derived from incandescent and fluorescent (Sylvania Cool White 215W) lamps. Composition of nutrient solutions was: 4.2 NO_3-N, 0.4 NH_4-N, 2.0 Ca, 1.0 K, 1.0 S, 0.5 Mg, and 0.1 P (in mM), and 20 Fe-EDDHA {ferric N,N'-ethylene bis[2-(2-hydroxy-phenyl) glycine]}, 9.4 Cl, 6.6 B, 4.7 Mn, 0.6 Zn, 0.2 Cu, 0.2 Na, and 0.1 Mo (in μM). To keep the nutrient composition similar

for the four plant species, Fe-EDDHA was used as the Fe source for all species. FeEDDHA as the Fe source for maize caused no Fe-deficiency symptoms to appear during growth at Ni levels from 0 to 120 μM.

White clover seeds were scarified with sand paper, sterilized with 0.50% NaOCl (household bleach; 1 NaOCl:10 water, V:V) for five minutes and thoroughly rinsed with distilled water. Seeds of the other plant species were likewise sterilized. Sterilized seeds were germinated in rolled germination filter papers using aerated 0.1-strength nutrient solution. Once germinated and of sufficient size, seedlings were transferred to containers with full-strength nutrient solutions and grown to obtain additional growth prior to introduction to the treatment solutions. Days from germination to the time plants were introduced to Ni treatments were 41 for white clover, 28 for ryegrass, 27 for cabbage, and 18 for maize. The number of plants of each species transferred to 2-L containers for application of the Ni treatments was 20 for white clover, 30 for ryegrass, 15 for cabbage, and 5 for maize. Nickel treatments were: 0, 15, 30, 60, 120, 240, and 320 μM added as $Ni(NO_3)_2$. Once plants were introduced to the treatments, the pH of nutrient solutions was maintained daily at 5.5. Plants were grown in the Ni treatments for 14 days. Treatments were randomized with three replications.

Plant Analysis and Root Length Measurement

Plant samples were harvested at the start and end of the Ni treatment periods. At harvest, roots of intact plants were immersed in 20 mM Na_2-EDTA (disodium ethylenediaminetetraacetate) for 15 min to remove Ni adhering to root surfaces. Roots and stalk bases were thoroughly rinsed with bi-distilled water, blotted dry, and shoots separated from roots. Shoots were dried at 65°C in a forced-air oven and weighed. Roots were cut into 1-2 cm segments, mixed thoroughly, and ~2 g representative fresh samples collected for root length measurements using a Comair root length scanner (Commonwealth Aircraft Corp., Melbourne, Australia[3]). The remainder of roots was dried similar to shoots and weighed. Dried shoot and root samples were ground to pass 0.5-mm stainless steel screen for chemical analysis. Samples (100 mg) were weighed into plastic containers, nitric acid (1.0 mL 15.6M HNO_3) added, containers placed in Parr microwave acid digestion bombs (Parr Instrument Co., Moline, IL[3]), microwaved for 4 minutes at 70% power, cooled, transferred to new containers, diluted to 10.0 mL with bi-distilled water, and

3. Mention of particular companies or commercial products does not imply recommendations or endorsement by USDA-ARS or VPI & SU over companies or products not mentioned.

filtered. Nickel concentrations were determined by graphite furnace atomic absorption spectrometry (Perkin Elmer, Norwalk, CT) when at very low levels and by inductively coupled plasma emission spectrometry (Applied Research Laboratories, Dearborn, MI[3]) against known standard solutions. National Institute of Standards and Technology Reference Plant Materials (NIST-SRMs) were also used to reference Ni determinations.

Calculations of Plant Growth and Nickel Influx and Transport

Plant growth rate constants (GRC), Ni influx (IN) rates into roots, and Ni transport (TR) rates from roots to shoots were calculated (Baligar et al., 1993; Verkleij and Parest, 1989) by using the following formula:

$$GRC = (InFW_2 - InFW_1) / (t_2 - t_1) \quad [1]$$

$$IN = [(PU_2 - PU_1) / (t_2 - t_1)] \times [(InRL_2 - InRL_1) / (RL_2 - RL_1)] \quad [2]$$

$$TR = [(SU_2 - SU_1) / (t_2 - t_1)] \times [(InSW_2 - InSW_1) / (SW_2 - SW_1)] \quad [3]$$

where: **FW** = fresh weight of whole plant (g/plant), **t** = time (days) of sampling at the start (subscript 1) and end (subscript 2) of Ni treatments, **PU** = Ni concentration in whole plant (mmol/plant), **RL** = root length (m/plant), **SU** = Ni concentration in shoots (mmol/plant), and SW = shoot dry weight (g/plant). **GRC** was reported as g FW/plant/d, **IN** was converted to pmol/cm RL/s, and **TR** was converted to nmol/g SW/s.

RESULTS

Nickel Toxicity Symptoms

Typical Ni toxicity symptoms on ryegrass young leaves including fully expanded new leaves were an uniform chlorosis with necrosis in the middle parts of leaf blades, while older leaves turned reddish-brown with mottling appearing on leaf blades, growth of new roots was completely inhibited, and roots had black root tips. Cabbage young leaves grew slowly, had chlorosis between leaf veins, black spots (dead tissue) developed later on the leaves, older leaves became dark green with purple color on the back of leaves, and leaves curled and wilted. White clover young leaves were uniformly chlorotic, older leaves turned yellowish-brown between leaf veins, and reddish-brown mottled spots appeared on the older leaf blades. Maize leaves wilted and died under severe toxicity (240-320 μM), and new leaves were slightly chlorotic between leaf veins when plants were grown with 120 μM Ni for about 10 days.

TABLE 1. Severity of Ni toxicity symptoms on four plant species after Ni treatment for 14 days.

Ni Level (μM)	Severity of Visual Toxicity Symptoms[1]			
	Maize	White-clover	Ryegrass	Cabbage
0	-	-	-	-
15	-	-	-	+
30	-	-	+	++
60	-	+	++	+++
120	+	+++	++++	++++
240	+++	++++	+++++	++++
320	+++	+++++	+++++	+++++

1. Rating of symptoms (% of leaves exhibiting toxicity symptoms): - = none; + = 0 to 10; + + = 10 to 30; + + + = 30 to 50; + + + + = 50 to 70; and + + + + + = >70.

Visible Ni toxicity symptoms appeared on shoots of ryegrass when plants were grown with 120 to 320 μM Ni after only one day, and severe toxicity symptoms developed when plants were grown with 30 to 240 μM Ni within six days. Toxicity symptoms were observed on cabbage grown with Ni levels >120 μM within four days. Visible toxicity symptoms on maize and white clover occurred when plants had grown with 240 to 320 μM Ni for five days. After 14 days treatment with Ni, toxicity symptoms on cabbage and ryegrass were visible at external Ni levels of 15 to 30 μM, whereas visible toxicity symptoms on white clover and maize were observed only at external Ni levels from 60 to 120 μM (Table 1). For plants grown with >120 μM Ni, the four species became sensitive to Ni, and Ni toxicity symptoms appeared. Each of the plant species developed Ni toxicity symptoms at shoot Ni concentrations of 50 to 80 μg/g.

Growth Responses

Maize and white clover whole plant DM increased slightly when grown at Ni levels <60 μM, whereas DM of ryegrass and cabbage significantly decreased when plants were grown at similar Ni levels (Fig. 1A). Decreases in DM were relatively large for maize and small for white clover when Ni level exceeded 120 μM. The GRC of each plant species decreased sharply with increasing Ni level (Fig. 1B). At

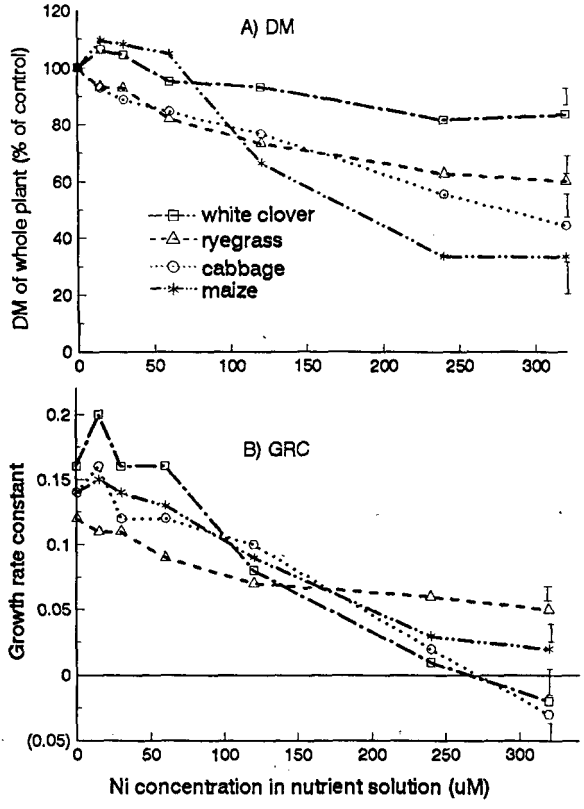

FIGURE 1. Dry matter (DM) and growth rate constant (GRC, g FW/plant/day) of four plant species grown with different levels of Ni in nutrient solution. The vertical bars are LSD ($P<0.05$) values for the corresponding data line. The DM for controls of the plant species was 0.81, 0.37, 0.89, 3.21 g/plant for white clover, ryegrass, cabbage, and maize, respectively.

Ni levels <120 μM, GRC of white clover was relatively high, while ryegrass had a relatively low GRC. The GRC was small for white clover and large for ryegrass when Ni level was increased >120 μM.

Nickel Influx and Transport

The IN of Ni increased with increasing Ni level for each species except maize, which decreased at Ni >120 μM (Fig. 2A). Increasing Ni from 0 to 120 μM, IN of

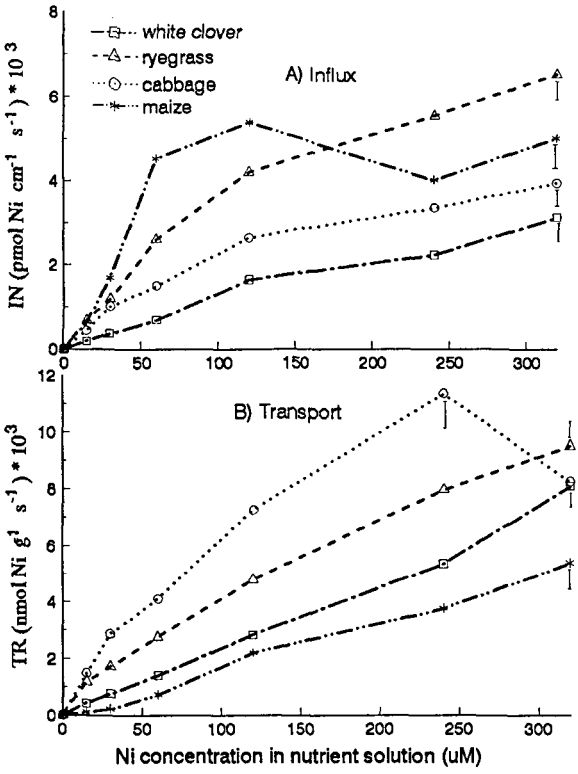

FIGURE 2. Nickel influx (IN) into roots and transport (TR) from roots to shoots of four plant species grown with different levels of Ni in nutrient solution. The vertical bars are LSD ($P<0.05$) values for the corresponding data line.

Ni by the four species followed the order of maize > ryegrass > cabbage > white clover. The IN of Ni was enhanced by ~7-fold for maize, ~5-fold for ryegrass, ~3-fold for cabbage, and ~2-fold for white clover when Ni in solution increased from 15 to 120 μM. Maize had relatively low TR of Ni, and cabbage had high TR of Ni (Fig. 2B). When grown with Ni <60 μM, TR of Ni for maize was very low compared to ryegrass and cabbage. Cabbage had relatively high TR of Ni at all Ni treatment levels, except at 320 μM where lethal toxicity occurred.

Plant Nickel Concentration

Nickel concentrations in shoots of each species increased with increasing Ni level (Fig. 3A). Cabbage had relatively high Ni concentrations in shoots, while

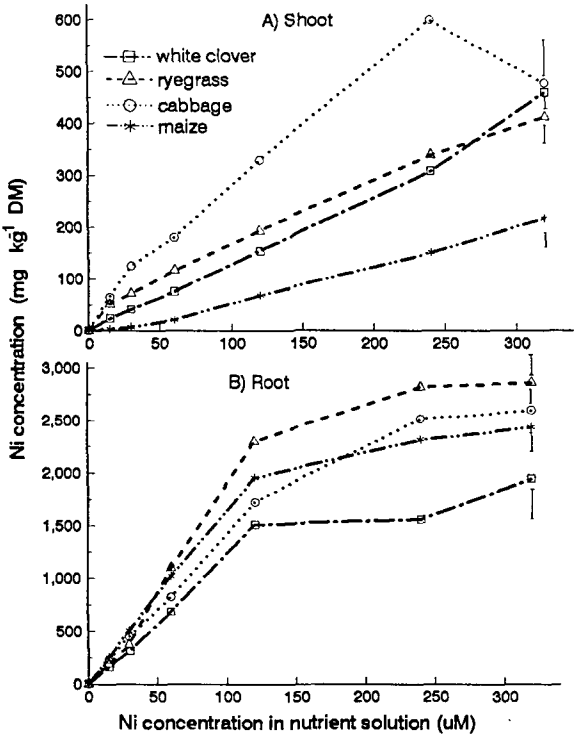

FIGURE 3. Nickel concentrations in shoots and roots of four plant species grown with different levels of Ni in nutrient solution. The vertical bars are LSD ($P<0.05$) values for the corresponding data line.

maize had relatively low Ni concentrations in shoots at all Ni levels. Nickel concentrations in roots of the four species increased sharply and linearly at <120 μM Ni in solution before increasing more gradually as Ni increased further (Fig. 3B). White clover had relatively low and ryegrass had relatively high root Ni concentrations when plants were grown with Ni >60 μM. Shoot/root Ni content ratios also differed among the species (Fig. 4). Cabbage was high while maize had low shoot/root Ni ratios. When Ni in the nutrient solution was between 0 and 30 μM, shoot/root Ni content ratios decreased sharply in all species except ryegrass, and especially sharp for maize.

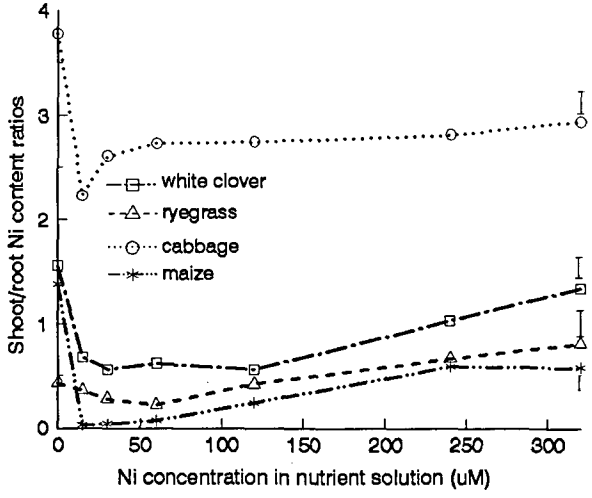

FIGURE 4. Shoot/root Ni content ratios of four plant species grown with different levels of Ni in nutrient solution. The vertical bars are LSD ($P<0.05$) values for the corresponding data line.

Nickel Tolerance Relationships to Nickel Influx and Transport

Decreases in GRC and DM were significantly and negatively correlated with IN, TR, and concentrations of Ni in shoots and roots (Table 2). The growth traits for maize were correlated with TR of Ni to shoots compared to IN of Ni into roots. High correlation between GRC and IN of Ni was also noted for cabbage.

DISCUSSION

Differences in accumulation of Ni among the plant ecotypes have been extensively investigated (Baker et al., 1985; Brooks et al., 1981). Many Ni-hyper-accumulator plants have been found to accumulated Ni at concentrations >1,000 µg/g without affecting normal growth when grown on serpentine soils (Baker et al., 1985; Brooks et al., 1977; 1979; Reeves, 1988; Reeves et al., 1983). Plant species and cultivars are also known to differ in their tolerance to Ni (Bingham et al., 1986; Farago and Cole, 1986; Foy et al., 1978; *Piccini and Malavolta*, 1992). In our study, we found shoot growth of maize and white clover to be enhanced slightly, but inhibited for ryegrass and cabbage when plants were grown with <60

TABLE 2. Correlation coefficients of growth rate constants (GRC) and dry matter (DM) with influx (IN) into roots, to shoots, concentrations of Ni in shoots (SNiC) and roots (RNiC), and shoot/root Ni content ratios (S/R-Ni) grown in nutrient solutions (n = 21).

	GRC				Decrease in DM (% of control)			
Ni trait	**Maize**	**White-clover**	**Ryegrass**	**Cabbage**	**Maize**	**White-clover**	**Ryegrass**	**Cabbage**
IN	-0.687*	-0.958**	-0.996**	-0.908**	-0.654	-0.910**	-0.996**	-0.969**
TR	-0.983**	-0.973**	-0.977**	-0.788*	-0.962**	-0.897**	-0.983**	-0.898**
SNiC	-0.981**	-0.975**	-0.978**	-0.854*	-0.956**	-0.899**	-0.978**	-0.940**
RNiC	-0.938**	-0.894**	-0.993**	-0.904**	-0.926**	-0.896**	-0.988**	-0.781*
S/R-Ni	-0.983**	-0.444	-0.763*	-0.092	-0.351	-0.370	-0.754*	-0.097

*and ** = significance at P = 0.05 and P = 0.01, respectively.

μM Ni for 14 days (Fig. 1A). Reasons for the enhanced growth of maize and white clover at 15 to 30 μM Ni are unknown, but these species may have had a moderate requirement for Ni or these species may have greater tolerance at these levels of Ni as compared to that for cabbage and ryegrass. The essentiality of Ni for plants was first reported for legumes (Eskew et al., 1983, 1984). Nickel levels in nutrient solution, whereby root DM was 70% that of the control, was ~150 μM for white clover, ~80 μM for maize, and 50 μM for ryegrass and cabbage. Nickel toxicity symptoms were observed on ryegrass and cabbage when plants were grown with Ni levels of 15 to 30 μM, but Ni toxicity symptoms appeared on maize and white clover only when plants were grown with 60 to 120 μM. Our results were consistent with the results observed in other studies in which effective dosage rates of Ni produced 50% of the normal DM, rates that were 3.1 μM for ryegrass, 6.8 μM for maize (Craig, 1978), and 85.2 μM for alfalfa (*Medicago sativa* L.) (Farago and Cole, 1986).

Tolerance of specific plant species to Ni toxicity was closely related to IN and TR of Ni in our study, especially TR of Ni. When plants were grown at moderately toxic levels of Ni (<120 μM), the relatively high tolerance of white clover to Ni toxicity was associated with relatively low IN and TR of Ni (Fig. 2). The relatively

high sensitivity of ryegrass to Ni toxicity was associated with high IN and TR of Ni. The tolerance of maize to Ni toxicity appeared to be associated mainly with its extremely low TR of Ni, and the sensitivity of cabbage to Ni toxicity appeared to be associated with its extremely high TR of Ni. In addition, GRC has been used as an index for heavy metal tolerance in plants (Verkleij and Parest, 1989). The GRC of ryegrass and white clover were highly correlated with low TR of Ni in our study (Table 2). Nickel tolerance of the plant species used in our study was also highly correlated with TR and accumulation of Ni in both shoots and roots.

Shoot/root Ni content ratios differed among plant species, high for cabbage whereas maize had low shoot/root Ni content ratios (Fig. 4). At Ni between 0 and 30 μM in the nutrient solution, shoot/root Ni content ratios decreased sharply in all species except ryegrass. This decrease was especially sharp for maize. These results might indicate that at moderately toxic Ni levels, ryegrass had rapid IN of Ni into roots and Ni was rapidly transported from roots to shoots, whereas Ni accumulation by maize was confined mainly to the roots. Thus, TR of Ni appeared to be a limiting factor for plant tolerance to Ni toxicity which might be used for selecting Ni-tolerant genotypes. The results of our study showed that under moderately toxic Ni conditions, TR of Ni may be important in identifying tolerance of plants to Ni toxicity. Therefore, selecting or developing genotypes with low TR of Ni could potentially improve the tolerance of plants to moderate Ni toxicity, and reduce the flow of Ni from contaminated soils to shoot organs. Good relative differences in IN (Fig. 2A), TR (Fig. 2B), and accumulation (Figures 3 and 4) of Ni among the four plant species were evident in our study, and significant correlations were obtained between Ni tolerance and IN, TR, and Ni accumulation (Table 2). Reasons for these differences are unknown, and the mechanisms of plant tolerance to Ni toxicity need to be better understood.

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